

# STRUCTURE OF THE ENERGY LANDSCAPE OF SHORT PEPTIDES

HANDAN ARKIN and TARIK ÇELİK<sup>†</sup>

*Hacettepe University, Physics Department, Ankara, Turkey*

<sup>†</sup> *E-mail: tcelik@hacettepe.edu.tr*

## Abstract

We have simulated, as a showcase, the pentapeptide Met-enkephalin (Tyr-Gly-Gly-Phe-Met) to visualize the energy landscape and investigate the conformational coverage by the multicanonical method. We have obtained a three-dimensional topographic picture of the whole energy landscape by plotting the histogram with respect to energy(temperature) and the order parameter, which gives the degree of resemblance of any created conformation with the global energy minimum (GEM).

*Keywords:* Energy Landscape, Conformational Sampling, Multicanonical Simulation.

# 1 Introduction

Biological macromolecules such as proteins have a well defined 3D structure which is essential for their biological activity. Therefore, predicting the protein's structure by theoretical/computational methods is an important goal in structural biology. [1]

The configuration space of peptide's and protein's presents a complex energy profile consisting of tremendous number of local minima; their basins of attraction were called localized microstates. The energy profile also contains larger potential energy wells defined over wide microstates (e.g., the protein's fluctuations around its averaged structure), each including many localized ones. [2]

Because of energy barriers, the commonly used thermodynamic simulation techniques, such as the Metropolis Monte Carlo (MC) [3] and molecular dynamics (MD) [4] are not very efficient in sampling a rugged landscape. Thus, the molecule remains in its starting wide microstate or move to a neighbor wide microstate, but in practice will hardly reach the most stable one. The system may occur to be trapped in a basin for a long time, which results in non-ergodic behavior. Therefore, developing simulation methods that lead to

an efficient crossing of the energy barriers has been a long standing challenge.

The topography of the energy landscape, especially near the global minimum is of particular importance, because the potential energy surface defines the behavior of the system. Methods for searching energy surfaces are proposed [5], energy landscape perspectives are investigated [6] and the fractal dimensions are studied [7]. The essence of a funnel structure of energy landscape at some fixed temperatures has recently been shown by Hansmann and Onuchic [8]. Consequently, a visualization of the whole rugged landscape covering the entire energy and temperature ranges would be helpful to develop methods allowing one to survey the distribution of structures in conformational spaces. Such a goal can be achieved within the multicanonical ensemble approach.

An ideal simulation scheme should freely visit the entire configuration space and predominantly sample the significant conformations. The trapping problem of the MC and MD methods can be alleviated to a large extent, by the multicanonical MC method (MUCA) [9, 10, 11], which was applied initially to lattice spin models and its relevance for complex systems was first noticed in Ref. [10]. Application of the multicanonical approach to peptides

was pioneered by Hansmann and Okamoto [12] and followed by others [13]; simulations of protein folding with MUCA and related generalized ensemble methods are reviewed in Refs. [14] and [15].

## 2 The Model

The multicanonical ensemble based on a probability function in which the different energies are equally probable. However, implementation of MUCA is not straightforward because the density of states  $n(E)$  is unknown *a priori*. In practice, one only needs to know the weights  $\omega$ ,

$$w(E) \sim 1/n(E) = \exp[(E - F_{T(E)})/k_B T(E)]. \quad (1)$$

These weights are calculated in the first stage of simulation process by an iterative procedure in which the temperatures  $T(E)$  are built recursively together with the microcanonical free energies  $F_{T(E)}/k_B T(E)$ , up to an additive constant. The iterative procedure is followed by a long production run based on the fixed  $w$ 's where equilibrium configurations are sampled. Re-weighting techniques (see Ferrenberg and Swendsen [16] and literature given in their second reference) enable one to obtain Boltzmann averages of various thermodynamic properties over a large range of temperatures.

As pointed out above, calculation of the *a priori* unknown MUCA weights is not trivial, requiring an experienced human intervention. For lattice models, this problem was addressed in a sketchy way by Berg and Çelik [10] and later by Berg [17]. An alternative way is to establish an automatic process by incorporating the statistical errors within the recursion procedure. The automatic procedure was tested successfully [18] as applied to models of the pentapeptide Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) described by the ECEPP/2 potential energy function [19].

In this work, as in our previous one, [18] Met-enkephalin is modeled by the ECEPP/2 potential, which assumes a rigid geometry, and is based on non-bonded, Lennard-Jones, torsional, hydrogen-bond, and electrostatic potential terms with the dielectric constant  $\epsilon = 2$ . This potential energy is implemented into the software package SMMP [21]. We further fix peptide bond angles  $\omega$  to their common value  $180^\circ$ , which leaves us with 19 dihedal angles as independent degrees of freedom (  $n_F = 19$  ). We have also simulated Met-enkephalin with variable peptide bond angles, for which the distribution of conformations are included in Table I.

### 3 Results and Discussions

We first carried out canonical (i.e., constant  $T$ ) MC simulations at the relatively high temperatures and MUCA test runs which enabled us to determine the required energy ranges. Then we performed full simulation which cover the high temperature region up to  $T_{\text{max}} = 1000$  K reliably. The energy range was divided into 31 bins of 1 kcal/mol each, covering the range  $[20, -11]$  kcal/mol. The lowest energy encountered was  $-10.75$  kcal/mol and  $T_{\text{max}} = 1000$  K was also used above 20 kcal/mol. At each update step, a trial conformation was obtained by changing *one* dihedral angle at random within the range  $[-180^\circ; 180^\circ]$ , followed by the Metropolis test and an update of the suitable histogram. The dihedral angles were always visited in a predefined (sequential) order, going from Tyr to Met; a cycle of  $N$  MC steps ( $N=19$ ) is called a sweep. The weights were built after  $m = 100$  recursions during a long *single* simulation, where the parameters  $b_i$  and  $a_i$  were iterated every 5000 sweeps.

For peptides it is not only of interest to obtain thermodynamic averages and fluctuations at different temperatures but also to find the most stable regions in conformational space populated by the molecule. In the organic

chemistry community conformational search methods have been developed and attempts have been made to find the global energy minimum (GEM) and all the energy minimized conformations in certain energy ranges above the GEM (see Ref.2(b), and references cited therein).

The lowest energy conformation (our suspected GEM) was found at  $E = -10.75$  kcal/mol.

Here we define, following Hansmann et. al. [8], an order parameter (OP)

$$OP = 1 - \frac{1}{90 n_F} \sum_{i=1}^{n_F} |\alpha_i^{(t)} - \alpha_i^{(RS)}|, \quad (2)$$

where  $\alpha_i^{(RS)}$  ve  $\alpha_i^{(t)}$  are the dihedral angles of the reference state (which is taken as GEM) and of the considered configuration, respectively. The difference  $\alpha_i^{(t)} - \alpha_i^{(RS)}$  is always in the interval  $[-180^\circ, 180^\circ]$ , which in turn gives for peptides

$$0 \leq \langle OP \rangle_T \leq 1 \quad (3)$$

Figure.1 shows the energy landscape obtained by the multicanonical simulation run of one million sweep plotted against energy and the order parameter. Here, we would like to point out that the utilized data is obtained by sampling of the conformational space and no minimization procedure is applied. At high temperatures, where the peptide is in the random coil state, the his-

togram looks as one gaussian-like peak centered around the value of the order parameter  $OP \sim 0.3$ . When the temperature is lowered, first a transition from the state of random coil to globular structure is expected. In Figure.2 we show the same energy landscape of Fig.1(b) by grouping the conformations of 1 kcal/mol interval in energy. Curve a) denotes the energy interval  $-1 \text{ kcal/mol} \leq E \leq 0 \text{ kcal/mol}$ , which corresponds after re-weighting to the temperature interval  $315 \text{ K} \leq T_a \leq 330 \text{ K}$ . At this temperature, the energy landscape starts deviating from a smooth surface and develops a shoulder. We identify this temperature as the starting of forming a structure rather than a random coil. Further down in energy (temperature), the newly developing branch becomes more populated. At the temperature  $215 \text{ K} \leq T_b \leq 230 \text{ K}$  denoted by the curve b), the energy landscape displays a typical structure bifurcating into two branches of almost equal height. From there on, the branch having larger values of the order parameter wins and more conformations populate that section of the conformational space. Our estimate of  $T_a$  and  $T_b$  from the topographic structure of the potential energy surface of Met-enkephalin are very close to the values of the collapse temperature  $T_\theta = 295 \pm 20 \text{ K}$  and the folding temperature  $T_f = 230 \pm 30 \text{ K}$ , respectively, determined by Hansmann et al [20]. We observe a third temper-



ature denoted by the curve c) in Fig.2 where the glassy behavior sets in and many valley structure of the landscape become clearly pronounced. For our simulated peptide sample Met-enkephalin, this temperature is in the range  $155 \text{ K} \leq T_c \leq 185 \text{ K}$ . Below this temperature, one observes the appearances of multiple valleys which are well separated by high energy barriers. The valley at the far-out end of the order parameter scale having the conformations with the value of the order parameter in the range  $0.98 \leq OP \leq 1$  contains the global energy minimum (GEM), respect to which the order parameter is evaluated. The temperature  $T_c$  seems to correspond to the glass transition temperature estimate of  $T_g = 180 \pm 30 \text{ K}$ , which value is based on the fractal dimension estimates. [7] In Fig.3 we plotted all the conformations found with energy  $E \leq -10.5 \text{ kcal/mol}$  with respect to the order parameter. Their number is 3587 conformations in one production run of one million sweeps. As clearly seen from Fig.3 that the conformations in this energy range are localized in one of the four valleys, which are identified by the value of their order parameter  $OP \sim 0.80, 0.87, 0.92$  and  $0.98$ . The conformations in the neighborhood of the GEM take place within the same wide microstate of the GEM but they are grouped into local microstates, each of which are one of the above mentioned valleys. The small differences in values of OP

comes from the differences in side-chain angles. We observe no conformation anywhere outside the definite valleys when the energy is less than about 1 kcal/mol above the GEM.

The number of conformations found in energy bins of 1 kcal/mol, which were plotted in Fig.2, appear in Table I. The lowest bin is 0.75 kcal/mol and includes the GEM. The table displays the distribution of sampled conformations according to the order parameter values, namely the distribution with respect to how far they are in configuration space from the global energy minimum. We also included in Table I the same distribution obtained in our simulation of Met-enkephalin for the case of variable peptide-bond angles  $\omega$ .

In conclusion, we have simulated the pentapeptide Met-enkephalin by utilizing the multicanonical ensemble approach and investigated the structure of the rugged energy landscape in the configurational space. We were able to display the distribution of at all temperatures from a single simulation and estimate the critical temperatures such as the collapse temperature, the folding temperature and the glass transition temperature. Such a visualization would be helpful in designing algorithms for efficient sampling of conformational space.

## 4 Acknowledgements

This work has been supported by the Hacettepe University Research Fund through project number 00.01.602.001. We are grateful to U.H.E. Hansmann for providing us the SMMP [21].

## References

- [1] M. Vásquez, G. Némethy, H.A. Scheraga, Chem Rev 94, 2183 (1994).
- [2] H. Meirovitch, E. Meirovitch, J Phys Chem 100, 5123 (1996); C. Baysal, H. Meirovitch, Biopolymers 50, 329 (1999).
- [3] N. Metropolis, A.W. Rosenbluth, M.N. Rosenbluth, A.H. Teller, E. Teller, J Chem Phys 21, 1087 (1953).
- [4] B.J. Alder, T.E. Wainwright, J Chem Phys 27, 1208 (1957); J.A. McCammon, B.R. Gelin, M. Karplus, Nature 267, 585 (1977).
- [5] S. F. Chekmarev, Phys. Rev. E 64, 36703 (2001); R. Elber, Curr. Opin. Struct. Biol. 6, 232 (1996); G. Némethy, H.A. Scheraga, Quart. Rev. Biophys. 10, 239 (1977); S. F. Chekmarev, S. V. Krivov, Phys. Rev. E 57, 2445 (1998).

- [6] J.N. Onuchic, Z. Luthey-Schulten, P.G. Wolynes *Annu. Rev. Phys. Chem* 48, 545 (1997); P.G. Wolynes, Z. Luthey-Schulten, J.N. Onuchic, *Chem Biol* 3, 425 (1996); J. D. Bryngelson, P. G. Wolynes, *Proc. Natl. Acad. Sci. USA* 84, 7524 (1987); Zh. Guo, Y. D. Thirumalai, *Biopolymers* 36, 103 (1995); J. E. Shea, Y. D. Nochomovitz, Zh. Guo, I. C. Brooks, *J. Chem. Phys.* 109, 2895 (1998).
- [7] D.A. Lidar, D. Thirumalai, R. Elber, R.B. Gerber, *Phys. Rev. E* 59, 2231 (1999); N.A. Alves and U.H.E. Hansmann, *cond-mat/0001195*.
- [8] U.H.E. Hansmann, Y. Okamoto, J.N. Onuchic, *Proteins* 34, 472 (1999); U.H.E. Hansmann, J.N. Onuchic, *J. Chem. Phys.* 115, 1601 (2001).
- [9] B.A. Berg, T. Neuhaus, *Phys Lett. B* 267, 249 (1991).
- [10] B.A. Berg, T. Çelik, *Phys Rev Lett* 69, 2292 (1992).
- [11] B.A. Berg, *Fields Institute Communications* 28, 1 (2000).
- [12] U.H.E. Hansmann, Y. Okamoto, *J Comput Chem* 14, 1333 (1993).
- [13] M.-H. Hao, H.A. Scheraga, *J Phys Chem* 98, 4940 (1994); *J Phys Chem* 98, 9882 (1994); A. Kolinski, W. Galazka, Skolnick, *J. Proteins* 26, 271

- (1996); J. Higo, N. Nakajima, H. Shirai, A. Kidera, H. Nakamura, J Comput Chem 18 ,2086 (1997).
- [14] U.H.E. Hansmann, Y. Okamoto, Ann. Rev. Comp. Physics 5, 129 (1999).
- [15] A. Mitsutake, Y. Sugita, Y. Okamoto, cond-mat/0012021, submitted to Biopolymers (Peptide Science).
- [16] A.M. Ferrenberg, R.H. Swendsen, Phys Rev Lett 61, 2635 (1988); Ibid 63, 1658 (1989).
- [17] B.A. Berg, Nucl Phys B (Proc. Suppl.) 63A-C, 982 (1998).
- [18] F. Yaşar, T. Çelik, B.A. Berg, H. Meirovitch, J Comp Chem 21, 1251 (2000).
- [19] F.A. Momany, R.F. McGuire, A.W. Burgess, H.A. Scheraga, J Phys Chem 79, 2361 (1975); M.J. Sippl, G. Némethy, H.A. Scheraga, J Phys Chem 88, 6231 (1984).
- [20] U.H.E. Hansmann, M. Masuya, Y. Okamoto, Proc. Natl. Acad. Sci. U.S.A. 94, 10652 (1997).

- [21] F. Eisenmenger, U.H.E. Hansmann, Sh. Hayryan, C.-K. Hu, [SMMP] A Modern Package for Simulation of Proteins, Comp. Phys. Comm (2001), in press.

Table 1: Number of conformations in energy bins of 1 kcal/mol.

ENERGY	OVERLAP				TOTAL
Fix $\omega$	1.0-0.9	0.9-0.8	0.8-0.7	0.7-0.6	CONF.
-10.75 to -10.0	3282	3935	3073	2779	15327
-10.0 to -9.0	1001	3530	4925	4475	28088
-9.0 to -8.0	467	2332	4003	3979	26220
-8.0 to -7.0	190	1460	3150	3488	24139
-7.0 to -6.0	90	897	2515	3290	22497
Variable $\omega$					
-12.21 to -12.0	23	25	-	-	48
-12.0 to -11.0	6380	7568	302	197	14457
-11.0 to -10.0	7600	21199	4775	2784	37107
-10.0 to -9.0	2700	9956	3959	3456	28430
-9.0 to -8.0	600	3107	2390	3137	2644

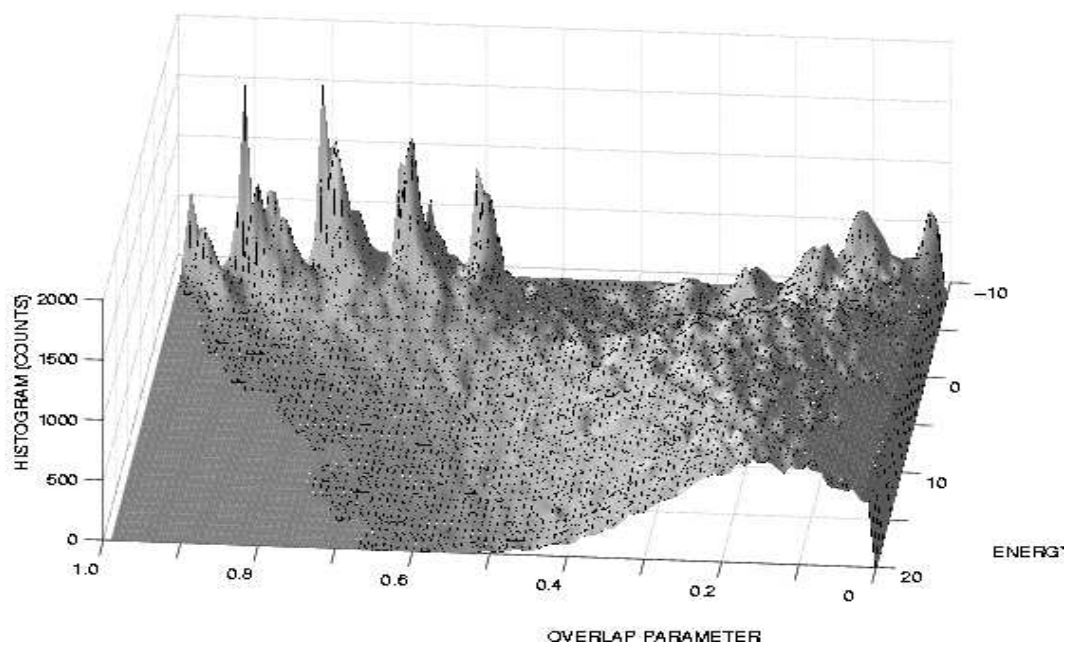
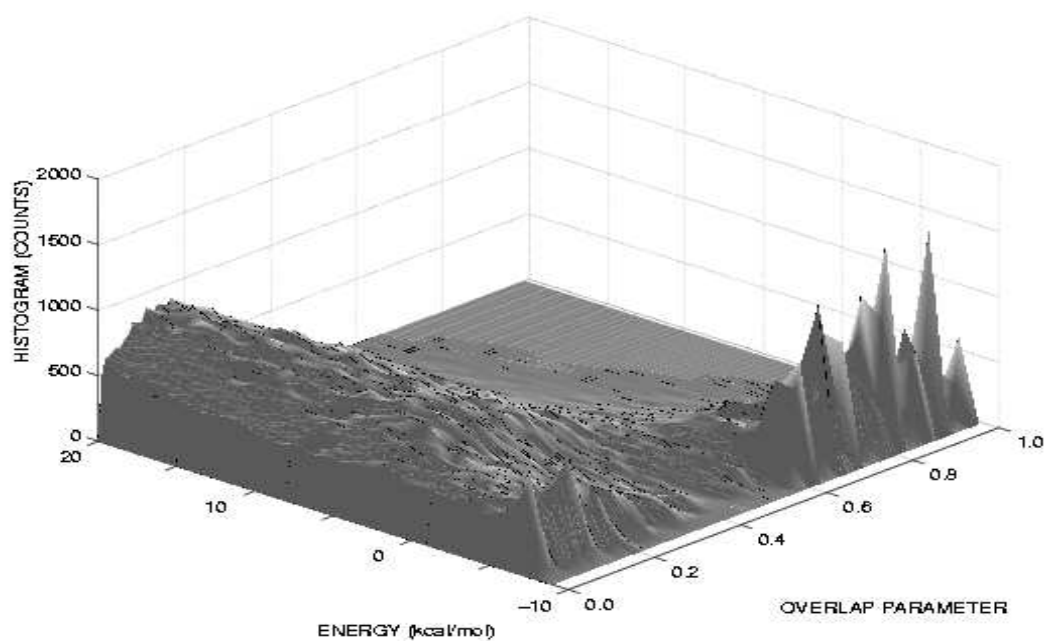


Figure 1: Energy surface in configuration space of Met-enkephalin viewed  
15  
from different angles.



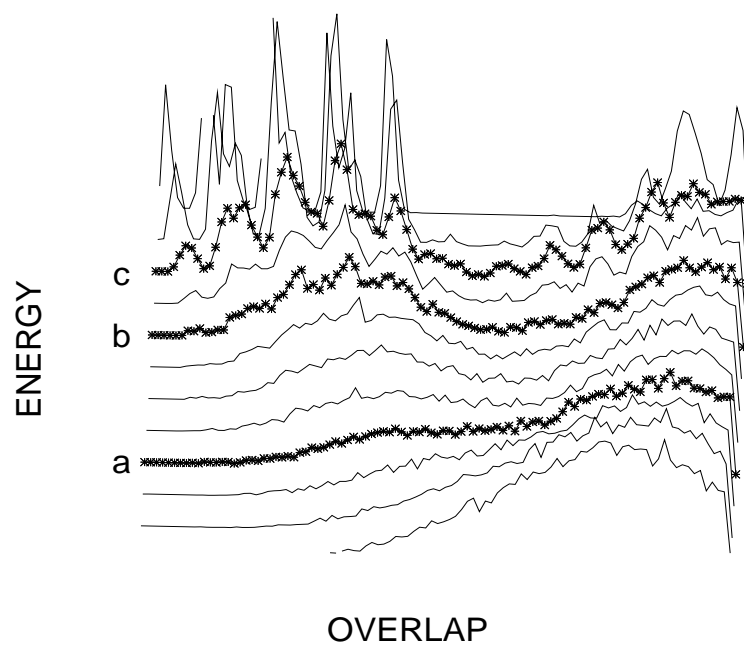


Figure 2: Same as Fig.1(b), plotted by grouping the conformations of 1 kcal/mol interval in energy.

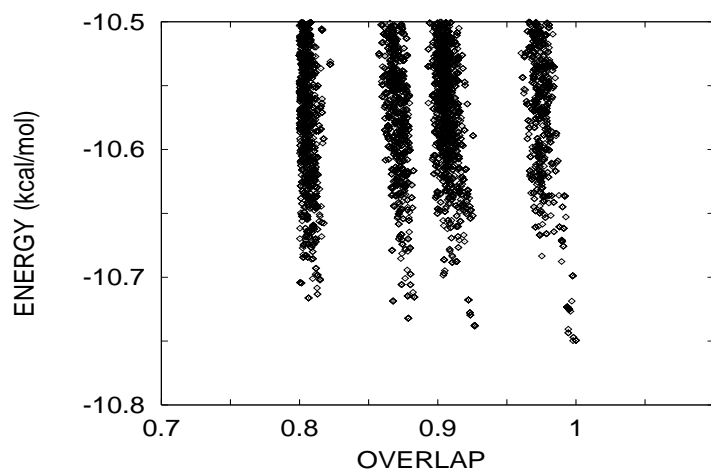


Figure 3: Distribution of microstates with  $E \leq -10.5$  kcal/mol with respect to the overlap parameter.